PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Nuijten et al.

Serial No.: 09/749,025

Filed: December 27, 2000

For: SALMONELLA VACCINE

Confirmation No.: 6121

Examiner: V. Ford

Group Art Unit: 1645

Attorney Docket No.: 2990-5048US

CERTIFICATE OF MAILING

I hereby certify that this correspondence along with any attachments referred to or identified as being attached or enclosed is being deposited with the United States Postal Service as First Class Mail on the date of deposit shown below with sufficient postage and in an envelope addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

April 27, 2007 — Date

Betty Vowles — Signature

Betty Vowles
Name (Type/Print)

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. PIET NUIJTEN

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dr. Piet Nuijten hereby declares:

1. I am a named inventor on the above-referenced patent application.

2. I am head of the department Bacteriological R&D and Program Manager of humane vaccine projects of Nobilon International B.V. A copy of my curriculum vitae is attached.

3. I understand that in the Office Action mailed October 20, 2006, the Examiner has questioned the fact that the application is enabling for a vaccine composition for the protection against Salmonellosis comprising an immunologically effective amount of any *Salmonella* mutated bacterium wherein the mutated bacterium lack flagellin and wherein the mutated bacterium is attenuated. I also understand that the claims at-issue have been amended and no

longer claim a "vaccine."

4. The as-filed specification is enabling because it includes working examples of non-flagellated mutant *Salmonella* compositions which reduce colonization rates in both chickens and pigs. Specifically, Example 3 of the specification demonstrates that chickens vaccinated with a non-motile mutant of *S. typhimurium* STMP, called *S. typhimurium* STM2000, had reduced colonization of the intestinal tract.

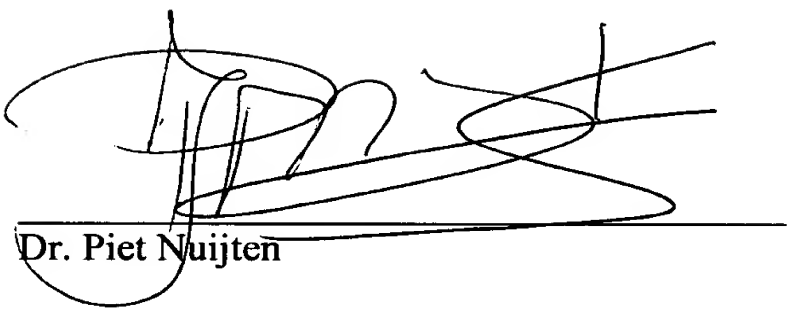
5. Example 4, at pages 22-23 of the specification, shows that the live attenuated flagella-less *S. typhimurium* STM2000 vaccine significantly reduced fecal shedding in pigs after a challenge infection with a wild-type *S. typhimurium* serotype.

6. The specification also provides detailed instructions for selecting non-motile mutants from serotype *S. typhimurium* SL3261. (Example 1, page 17). In this example, a flagellin protein gene of *S. typhimurium* SL3261 was chemically mutagenized with NTG and non-motile mutants were selected by light microscopy. The selected mutant was named STM2001 and subsequent electrophoretic analysis revealed that the mutant lacked the flagellin protein fragment of 51kDa and pI 4.7, as compared to the non-mutant parent serotype. *Id.*

7. Attached hereto, I present *in vivo* data using four different strains of *Salmonella enterica* bacteria (*S. typhimurium, S. enteritidis, S. anatum and S. hadar*) confirming reduced colonization of wild-type Salmonella in the cloacae of chickens after they were given *Salmonella enterica* fla^- strains. Therefore, it is submitted that the application provides the skilled person with sufficient guidance to make and use the claimed compositions.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: April 23, 2007



Dr. Piet Nuijten

Curriculim vitae P.J.M. Nuijten April 2007

Personal data.

Name:	Petrus Johannes Maria (Piet) Nuijten
Address:	Radioweg 1
City:	Sambeek
Zipcode:	5836CD
Country:	The Netherlands
Nationality:	Dutch
Place of birth:	Bergen op Zoom, The Netherlands
Date of birth:	October 5th 1959

Role:

Head of Department Bacteriological R&D and program manager of human vaccine projects, Nobilon International BV. Boxmeer, The Netherlands.

Academics:

1979-1987	Wageningen University, Netherlands: biochemistry, molecular virology, molecular bacteriology. 1985-1986: Master training period in University of Kentucky, Lexington, USA.
1987-1991	Veterinary Faculty, Utrecht University, Netherlands. Ph.D. Project: Molecular Pathogenesis of Campylobacter jejuni.
1991	Ph.D. Thesis
1993-1997	Supervisor Ph.D. Project. Veterinary Faculty, Utrecht University. M. Kolkman. Capsular polysaccharide synthesis in Streptococcus pneumoniae. Thesis Dec 4th 1997.
1994-2000	Supervisor Ph.D. Project.. Institute for Animal Sciences, Lelystad. A. Lammers Pathogenesis of Staphylococcus aureus mastitis. Thesis Oct 5th 2000.
2000-2004	Supervisor Ph.D. Project. Intervet International BV, Boxmeer. D. Schuijffel. A strategic approach for immunity-based selection of cross-protective Ornithobacterium rhinotracheale antigens. Thesis March 17th 2005.

Professional history:

1991-1993	Veterinary Faculty, Utrecht University, Netherlands.

	Post-doc Project: Biosynthesis pathways of capsule polysaccharides in Streptococcus pneumoniae.
1993-1994	Medical Faculty, Stanford University, Palo Alto, USA. Post-doc Project: Identification of toxins in Helicobacter pylori
1994-1996	Institute for Animal Sciences, Lelystad, Netherlands. Post-doc Project: Virulence mechanisms of Staphylococcus aureus in mastitis.
1996-2004	Intervet International BV, Molecular Bacteriology, Boxmeer, Netherlands. Animal vaccines. Project leader of many different projects: Salmonella, Haemophilus, Staphylococcus, Streptococcus, Campylobacter, Mycoplasma,
2004-	Nobilon International BV. Boxmeer, Netherlands. Human vaccines. Head Bacteriological R&D and Program manager of several vaccine projects.

Publications:

One page.

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UreA2B2: a second urease system in the gastric pathogen Helicobacter felis.
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7: Jacobs AA, Goovaerts D, Nuijten PJ, Theelen RP, Hartford OM, Foster TJ. Related Articles, Links

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9: Schijns VE, Weining KC, Nuijten P, Rijke EO, Staeheli P. Related Articles, Links

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Diversity of capsular polysaccharide synthesis gene clusters in Streptococcus pneumoniae.

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Capsular polysaccharide synthesis in Streptococcus pneumoniae serotype 14: molecular analysis of the complete cps locus and identification of genes encoding glycosyltransferases required for the biosynthesis of the tetrasaccharide subunit.
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Analysis of flagellin gene expression in flagellar phase variants of Campylobacter jejuni 81116.
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Differential flagellin expression in a flaA flaB+ mutant of Campylobacter jejuni.
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Localization of immunogenic regions on the flagellin proteins of Campylobacter jejuni 81116.
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Size and physical map of the Campylobacter jejuni chromosome.
Nucleic Acids Res. 1990 Nov 11;18(21):6211-4.
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22: Nuijten PJ, van Asten FJ, Gaastra W, van der Zeijst BA. Related Articles, Links

Structural and functional analysis of two Campylobacter jejuni flagellin genes.
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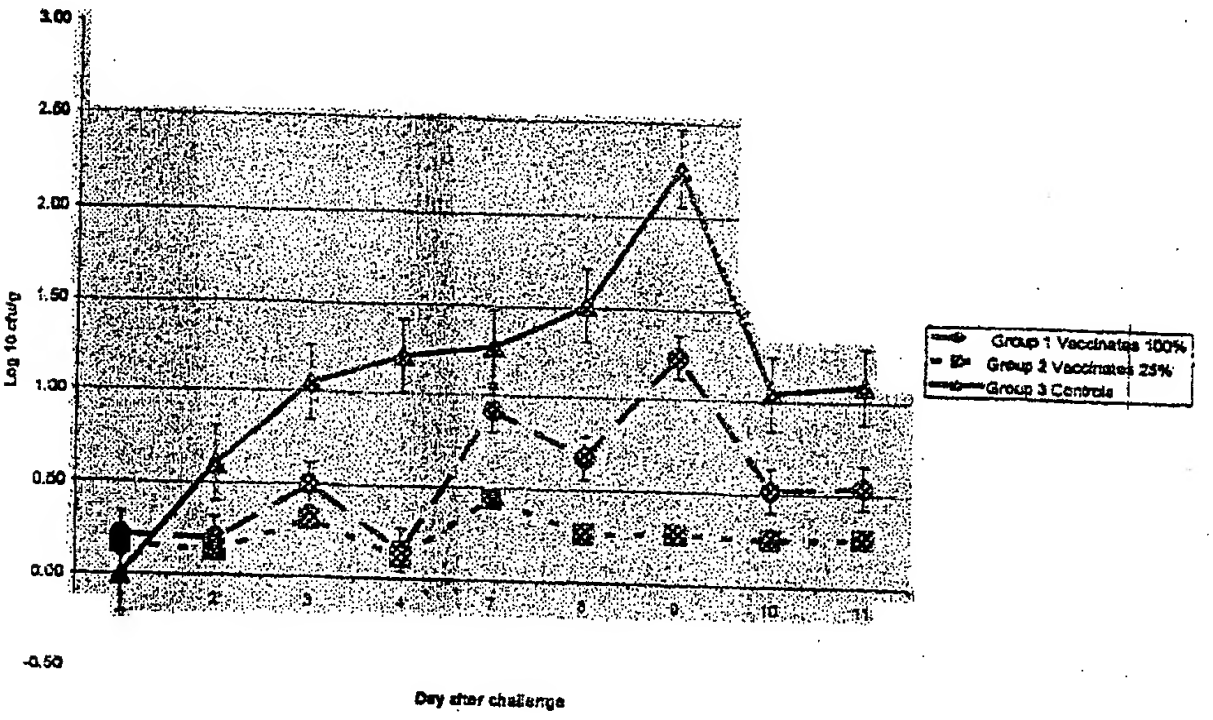
SUMMARY

Group 1 of 30 SPF layers were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising $2x10^9$ killed cells of each of FliC mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 3 (30 birds) was not vaccinated and Group 2 (30 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with 10^7 cfu of a different strain of wild type S. Anatum and this inoculation was repeated on the 2 subsequent days (i.e. 3 lots of 10^7 cfu over 3 days). Cloacal samples were tested for the presence of S. Anatum for 11 days. The extent of S. Anatum colonisation was expressed as the group mean number of S. Anatum cfu/g of cloacal sample. S. Anatum colonisation in group 1 (figure) was significantly less ($P<0.05$) than seen in group 3 (figure) or group 2. S. Anatum colonisation in group 2 was also significantly less than in group 3 (figure).

Figure 1. Numbers of S. Anatum recovered from cloacal swabs.



SUMMARY

Group 1 (28 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising $2x10^9$ killed cells of each of fliC mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 2 (28 birds) was not vaccinated and Group 3 (28 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with 10^9 cfu of a different strain f wild type S.Enteritidis. Cloacal samples were tested for the presence of S.Enteritidis for 14 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S.Enteritidis colonisation was expressed as the percentage of birds with different levels of contamination. S.Enteritidis colonisation in group 1 (figure 1) was significantly less ($P<0.05$) than seen in group 2 (figure 2) or group 3 (not shown: group 3 not significantly different form group 2).

Figure 1

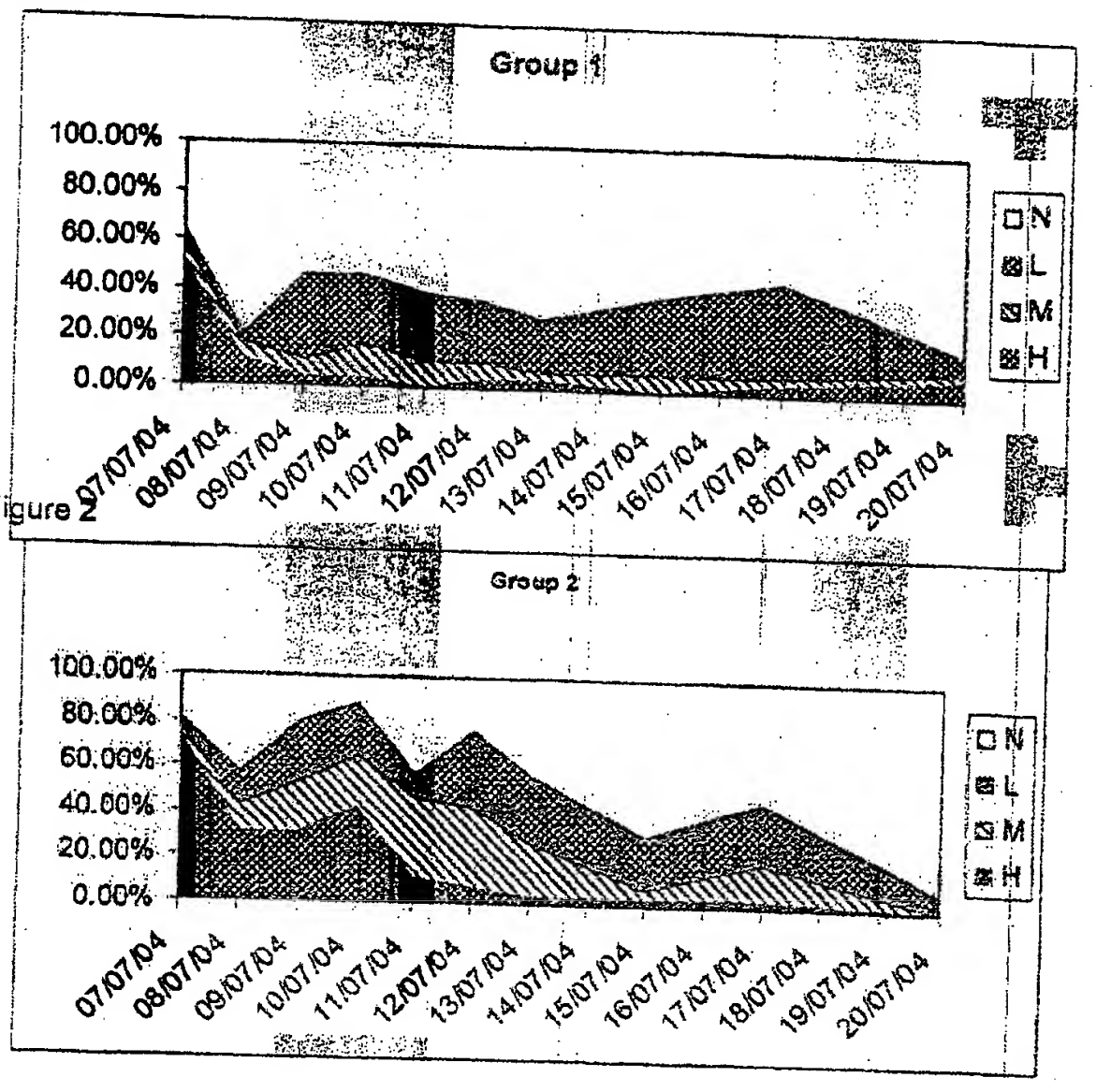


Figure 2

SUMMARY

Group 6 (28 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising $2x10^9$ killed cells of each of fliC mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 7 (28 birds) was not vaccinated and Group 8 (28 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with 10^9 cfu of a different strain of wild type S. Hadar. Cloacal samples were tested for the presence of S. Hadar for 14 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S. Hadar colonisation was expressed as the percentage of birds with different levels of contamination. S. Hadar colonisation in group 6 (figure 1) was significantly less ($P<0.05$) than seen in group 7 (figure 2) or group 8 (not shown: group 8 not significantly different form group 7).

Figure

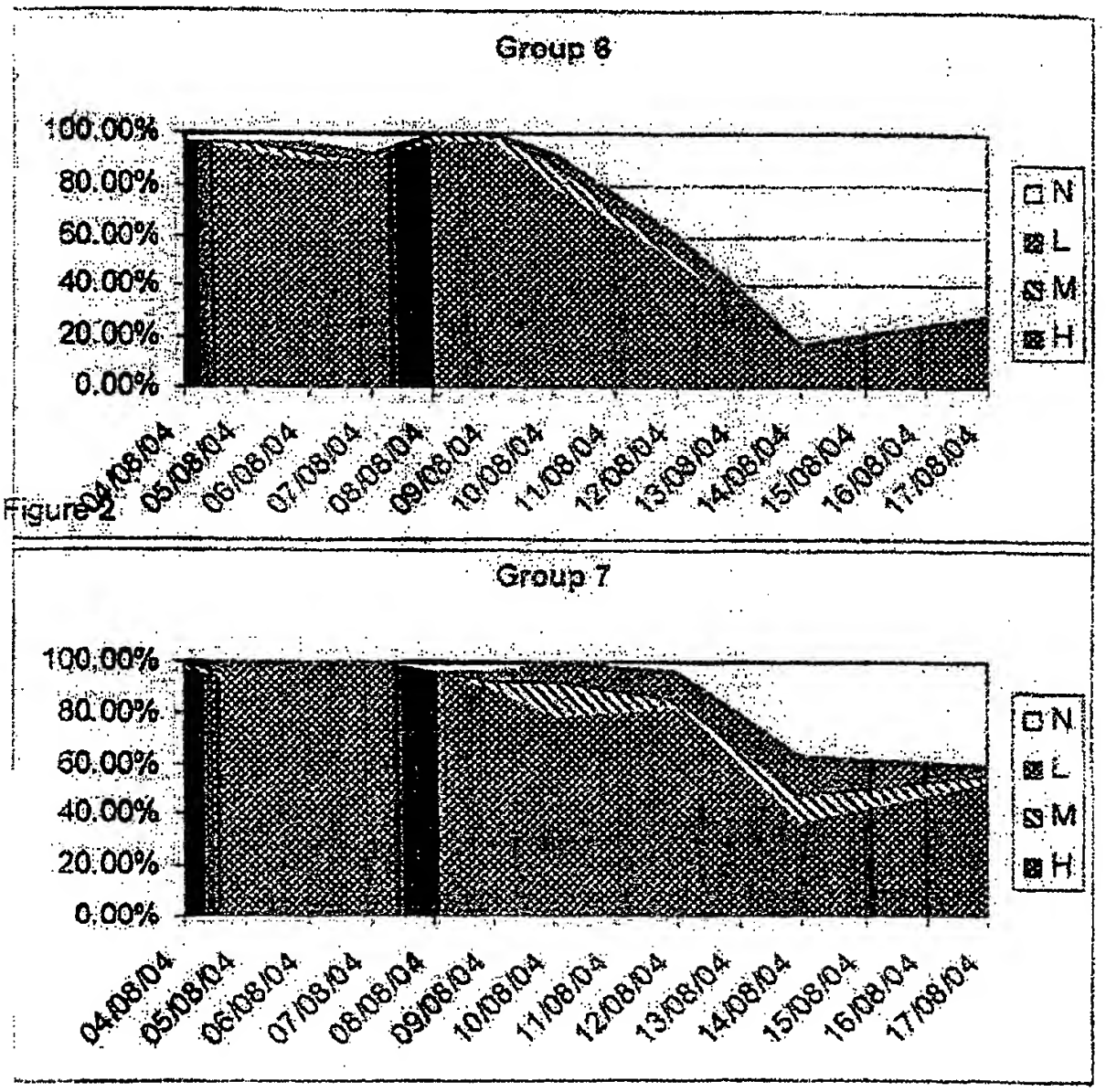


Figure 2

SUMMARY

Group 11 (14 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising $2x10^9$ killed cells of each of fliC mutants S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 12 (14 birds) was not vaccinated. At 12 weeks of age all birds were challenged orally with 10^9 cfu of a different strain of wild type S.Typhimurium and this inoculation was repeated on the 2 subsequent days (i.e. 3 lots of 10^9 cfu over 3 days). Cloacal samples were tested for the presence of S.Typhimurium for 11 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S.Typhimurium colonisation was expressed as the percentage of birds with different levels of contamination. S.Typhimurium colonisation in group 11 (figure 1) was significantly less ($P<0.05$) than seen in group 12 (figure 2).

Figure 1

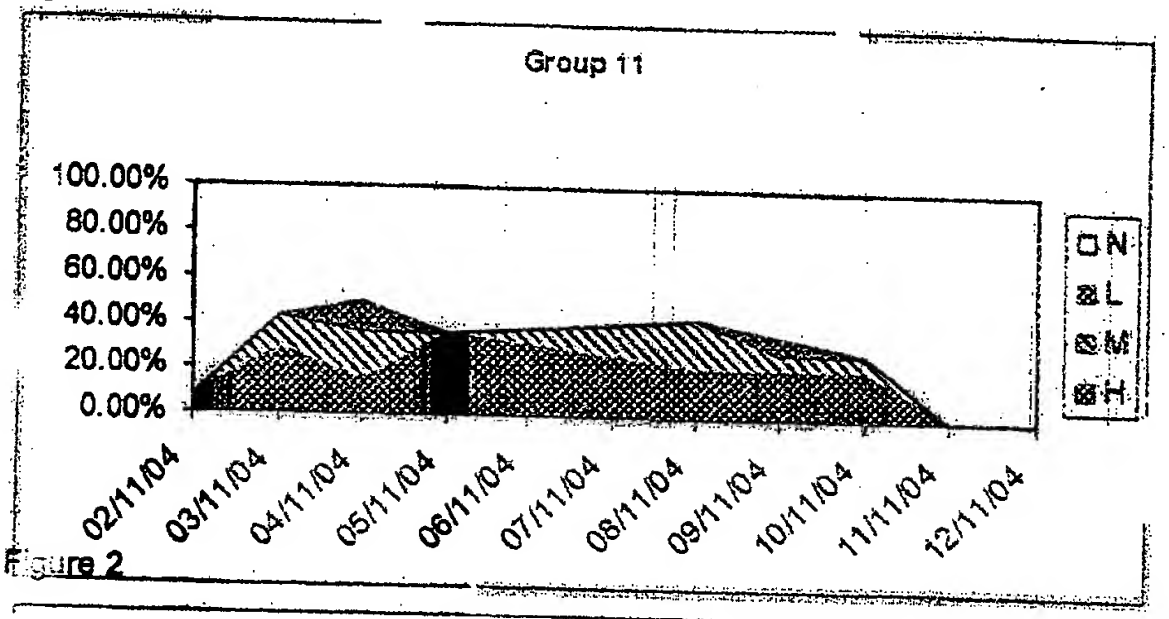


Figure 2